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ARTICLE

Insulin resistance and early virological response in chronic HCV infection

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KEYWORDS

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Abstract *Background:* Studies revealed that insulin resistance is associated with fibrosis progression and has negative impact on sustained virological response after standard antiviral therapy in patients with chronic hepatitis C (CHC).

Aim: To assess the role of IR on progression of liver fibrosis and early virological response (EVR) rates in patients with chronic hepatitis C infection.

Patients and methods: The study population comprised 79 subjects who underwent combination therapy for CHC. Laboratory investigations in the form of glucose, insulin, bilirubin, alanine aminotransferase (ALT), cholesterol and triglycerides and liver biopsy were done for all patients. Insulin resistance was calculated using the homeostasis model of IR (HOMA-IR).

Results: IR was increased (>2 IU) in 31 (40.7%) of patients. Early virological response was achieved among 37 patients (48.7%). No difference in EVR, viral load or grade of liver fibrosis between patients with and without IR. A significant positive correlation was found between IR and liver steatosis.

Conclusion: Insulin resistance is a common finding in CHC, it is associated with increase liver steatosis. However it has no impact on EVR to combined interferon ribavirin therapy, viral load or necroinflammation.

1. Introduction

Insulin resistance (IR) is a condition in which higher than normal insulin concentrations are needed to achieve normal metabolic responses, or in which normal insulin concentrations fail to elicit a normal metabolic response [1].

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Insulin resistance is considered the main underlying cause of metabolic syndrome, and the main pathogenetic factor for non alcoholic fatty liver disease (NAFLD), the hepatic manifestation of the metabolic syndrome [2].

Recently, several studies are focused on the relationship of insulin resistance and chronic hepatitis C (CHC). Different lines of evidence have found that IR is a common feature in patients with CHC especially with genotypes 1 and 4 [3,4]. Studies in animal models using genotype 1 constructs revealed that the development of IR occurred early and in the absence of liver injury or body weight gain, providing support for a direct link between CHC infection and IR [5]. The clinical relevance of IR in HCV arises from its ability to promote hepatic inflammation and fibrosis and to impair response to antiviral therapy [4,6].

However, Contrasting data exist on the role of IR as a predictor of sustained virological response (SVR) in the setting of both HCV mono-infected and HIV/HCV coinfected subjects [7–9].

The reference method for assessment of IR is the glucose clamp technique; however this method is expensive and laborious. The homeostasis model assessment (HOMA) of insulin sensitivity was proposed as a simple and inexpensive alternative and is a good reflection of that assessed by the glucose clamp technique [10].

EVR (Early Virological Response): EVR means that hepatitis C viral load has dropped by 99% (2logs), or is undetectable after 12 weeks of HCV treatment. An EVR is a good predictor of the ultimate response to HCV treatment. If a person does not have an EVR, their chance of SVR is very low (1–4%). Usually, HCV treatment is discontinued in people who do not have an EVR [11].

The aim of the study was to assess the effect of insulin resistance, measured by HOMA test for insulin sensitivity, on the early virological response to hepatitis C virus therapy in HCV infected patients.

2. Patients and methods

2.1. Patients

We prospectively evaluated 76 patients with chronic HCV infection recruited from National Liver and Tropical Disease Institute. Patients were subjected to detailed history and clinical examination. Liver biopsy and laboratory investigations were done for all patients before starting therapy. All patients were enrolled in the study after signing the informed consent and approval of ethical committee in national research center.

Patients were initiated on treatment with subcutaneous pegylated interferon alfa-2a (180 μg/week) plus oral ribavirin (1000 or 1200 mg/day).

Exclusion criteria were: patients with hepatitis B infection or human immunodeficiency virus infection, autoimmune or metabolic liver diseases, diabetes mellitus, patients with body mass index (BMI) $\geqslant 30\ kg/m^2$, patients not eligible for interferon therapy. Also we excluded patients who were previously treated by any drug that may affect the results.

The end point was to assess early virological response and its relation to IR. Early virological response (EVR) was defined as HCV RNA undetectable at week 12.

2.2. Laboratory assessment

Blood samples were collected after a 12-h overnight fast and deposited in dry tubes with EDTA. The plasma was separated immediately using refrigerated centrifugation at 2500–3000 rpm for a period of 10 min. The samples were processed after conservation at −20 °C. Blood was collected for the determination of the serum levels of plasma glucose, insulin and alanine aminotransferase (ALT), cholesterol, triglycerides were measured after precipitation with polyanions Plasma glucose was measured immediately on fresh samples collected in oxalate tubes. Serum insulin was determined by a radioimmunoassay (Phasdaseph Insulin RIA; Pharmacia and Upjohn Diagnostics AB, Uppsala, Sweden) [12].

Insulin resistance (IR) was calculated using the homeostasis model of IR (HOMA-IR).

HOMA-IR = fasting glucose (mmol/L) × fasting insulin (μU/ml)/22.5 [12,13]. Patients with HOMA IR > 2 were considered to have IR [14].

Hepatitis CV RNA was assessed before and 12 weeks after therapy. Viral load less than 10^6 copies/ml was considered mild viremia, between 10^6 and 10^8 copies/ml moderate viremia, more than 10^8 copies/ml severe viremia.

2.3. Histopathological examination

An ultrasound guided percutaneous liver biopsy was performed for all subjects. The degree of necroinflammatory activity and of fibrosis was scored by an expert hepatopathologist based on the Ishak score [15] (F0, no fibrosis; F1, portal fibrosis without septa; F2, portal fibrosis with rare septa; F3, numerous septa without cirrhosis; F4, cirrhosis). Necroinflammation scored from 6 to 14. Hepatic steatosis was scored as the percentage of hepatocytes containing macrovesicular fat droplets. The grading was conducted as follows: grade 0, no steatosis; grade 1, < 33% of hepatocytes affected; grade 2, 33–66% of hepatocytes affected; grade 3, > 66% of hepatocytes affected [16].

2.4. Statistical analysis

Data were presented as mean and standard deviation (SD) and percentage. The data were analyzed by SPSS version 14 (SPSS Inc., Chicago, IL, USA). The following tests of significance were used: *t*-test between means to analyze differences between

Table 1 Demographic and laboratory characteristics of the patients.

	Mean ± SD	Range
Age, years	47 ± 12	18–70
BMI, kg/m^2	25.6 ± 1.5	22.5–29.7
Waist,cm	84.6 ± 6.3	68–94
ALT, IU/dl	55.8 ± 48.9	10–250
Triglycerides, mg/dl	196.2 ± 121.6	135–123
Cholesterol, mg/dl	213.8 ± 24.4	159–373
FBG, mmol/L	4.9 ± 0.6	3.8–6.7
HOMA-IR	2.6 ± 5	0.2–12.8

BMI, body mass index; ALT, alanine aminotransferase; FBG, fating blood glucose; HOMA-IR, homeostasis model assessment; IR, insulin resistance.

Table 2 Difference between patients with and without insulin resistance.

Variables	Patients with IR ⩽ 2 $n = 45$	Patients with IR > 2 $n = 31$	p Value
Age, years, mean ± SD	43.3 ± 13.1	52.6 ± 8.1	< 0.001*
Sex n (%)			
Males ($n = 51$)	27(60)	24(77.4)	0.961
Females ($n = 25$)	18(40)	7(22.6)	
BMI, kg/m^2, mean ± SD	25.5 ± 1.6	25.7 ± 1.1	0.6
Waist, cm, mean ± SD	83.2 ± 6.8	86.6 ± 5.1	0.02*
ALT, IU/dl, mean ± SD	53.1 ± 52.9	56.5 ± 44.1	0.8
Triglycerides, mg/dl, mean ± SD	206.6 ± 159.0	180.1 ± 12.8	0.4
Cholesterol, mg/dl mean ± SD	216.3 ± 29.5	211.1 ± 14.6	0.4
FBG, mmol/dl, mean ± SD	4.9 ± 0.6	4.9 ± 0.7	0.8
Fasting insulin IU/dl, mean ± SD	4.6 ± 2.9	24.2 ± 13.8	< 0.001
Viral load n (%)			
• Mild viremia	17 (37.8%)	17 (54.8%)	
• Moderate viremia	18 (40%)	6 (19.4.0%)	0.2
• Marked viremia	10 (22.2%)	8 (25.8%)	
Necroinflammation mean ± SD	10.8 ± 2.1	10.9 ± 1.8	0.6
Steatosis n (%)			
⩽33%	9 (20%)	3 (9.7%)	0.2
> 33%	36 (80%)	28 (90.3%)	
Fibrosis, n (%)			
• Grades 1–2	14 (31.1%)	8 (26.7%)	0.7
• Grades 3–4	31 (68.9%)	23 (73.3%)	
EVR, n (%)			
• Responders	21 (43.3%)	16 (51.6%)	0.5
• Non responders	24 (56.7%)	15 (48.4%)	

BMI, body mass index; ALT, alanine aminotransferase; EVR, early virological response.

Table 3 Correlation between Insulin resistance and other parameters.

	Insulin resistance	
	r	p
Age	0.311	0.006
Weight	0.120	0.440
Height	0.048	0.881
BMI	0.097	0.242
Waist	0.127	0.244
ALT	0.007	0.890
Triglycerides	0.056	0.285
Cholesterol	0.061	0.463
FBG	0.016	0.642
Necroinflammation score	0.083	0.677
Steatosis	0.255	0.03*

BMI, body mass index; ALT, alanine aminotransferase; FBG, fating blood glucose.
* p Value is significant.

means Differences between nominal variables were analyzed by χ^2 tests. Two-tailed p-value < 0.05 was considered statistically significant.

3. Results

3.1. Patients characteristics

The current study comprised 76 patients with chronic HCV infection; they were 51 men and 25 women. Demographic and laboratory characteristics of the patients are presented in Table 1.

Before starting treatment viral load was mild (< 10^6 copies/ml) in 34 patients (44.7%), moderate (10^6–10^8 copies/ml) in 24 patients (31.6%) and severe (> 10^8 copies/ml) in 18 patients (23.7%).

Insulin resistance assessed by HOMA test ranged from 0.2 to 12.8 with mean of 2.6. Thirty one (40.7%) patients had insulin resistance > 2.

Histopathological evaluation of liver biopsies showed that the score of necroinflammation ranged from 6 to 14 with mean value of 10.7 ± 2.0, Liver steatosis was scored as the percentage of hepatocytes containing macrovesicular fat droplets, it ranged from 20% to 55% with mean of 41%. Grade1 steatosis was present in 12 (15.8%), grade 2 and 3 in 64 (84.2%). Liver fibrosis ranged from 1 to 3. Grades 1 and 2 fibrosis were present in 22 (28.9%) patients, grade 3 in 54 (71.1%) patients. Early virological response was achieved among 37 patients (48.7%).

We investigated the difference between patients with IR > 2 and patients with IR ⩽ 2: We found no significant difference in EVR, the difference was in the mean age and waist circumference. Percentage of liver steatosis that were higher in patients with IR > 2.

But this difference did not reach the significant value (Table 2).

We investigated the correlation between IR and studied parameters, and we found significant positive correlation with age, and degree of hepatic steatosis (Table 3).

We investigated the relation between necroinflammation and hepatic steatosis and we found that the mean value of percentages of liver steatosis were significantly higher among

Table 4 Comparison between responders and non responders.

	Non responders ($N = 41$)	Responders ($N = 35$)	$t^{\wedge}/\chi^{2\#}$	p
Age, years, mean ± SD	52.4 ± 9.6	42.0 ± 11.9	−4.235	<0.001*
Sex n (%)				
Males ($n = 51$)	28 (68.3%)	23 (37.1%)	0.52	0.471
Females ($n = 25$)	13 (31.7%)	12 (62.9%)		
BMI, kg/m^2, mean ± SD	26.1 ± 1.4	25.2 ± 1. 3	−3.020	0.003*
Waist cm, mean ± SD	84.4 ± 6.3	84.8 ± 6.5	−0.280	0.780
ALT IU/dl, mean ± SD	57.1 ± 49.1	54.3 ± 49.2	0.251	0.802
Triglycerides, mg/dl, mean ± SD	205.9 ± 170.9	187.2 ± 40. 4	−0.681	0.498
Cholesterol, mg/dl, mean ± SD	213.2 ± 17.8	214.5 ± 30.1	−0.241	0.810
IR	1.8 ± 0.3	1.5 ± 0.4	−0.463	0.643
Viral load				
• Mild	17 (41.5%)	17 (48.5%)		
• Moderate	16 (39.0%)	9 (25.7%)	1.524	0.467
• Marked	8 (19.5%)	9 (25.7%)		
Fibrosis, n (%)				
• Grades 1–2	8 (19.5%)	14 (40%)	3.808	0.051*
• Grades 3–4	33 (80.5%)	21 (60%)		
Grade of necroinflammation mean ± SD	11.1 ± 1.9	10.3 ± 2.1	1.666	0.100
Steatosis, %, mean ± SD	42.0 ± 8.1	40.1 ± 9.0	1.037	0.303

^ Independent t-test.
# Chi square test.
* p value is significant.

patients with grade 3 and 4 fibrosis (43.2 ± 7.8%) than patients with Grades 1 and 2 fibrosis (35.4 ± 8.1%) ($p = 0.0001$) and the score of necroinflammation Table 4 was directly correlated to liver steatosis ($r = 0.684$, $p = 0.0001$).

We studied the effects of the studied parameters on response to treatment and it was found that lower ages; lower BMI and lower grades of fibrosis were the only predictors for good EVR.

4. Discussion

In recent years, several studies reported association between IR and chronic HCV infection especially with genotype 1and 4 [3,4]. Insulin resistance has impact on the natural history of the disease and treatment outcomes, as it promotes hepatic inflammation and fibrosis and impairs rapid and sustained virological response rates to combined pegylated interferon/ribavirin therapy in chronic HCV [7,17–19]. The association of IR and CHC ranged between 30% and 70%, the higher association is with genotype 1 and 4 [20].

Given the association between IR and poor treatment response in CHC, clinical trials of insulin-sensitizing drugs have been proposed to improve treatment response [21]. So that, our aim was to identify the role of insulin resistance as a disease modifier affecting progression of liver fibrosis and early virological response in chronic HCV infection.

In the current study, IR was detected in 40.8% of our patients. Studying the impact of IR on EVR, we did not find significant difference in the rate EVR between patients with IR $\leqslant 2$ and patients with IR > 2; this was in contradiction with Eslam and his colleagues study who found that IR is a major determinant of the early viral kinetic response to peg interferon plus ribavirin, which has a great impact on subsequent rapid virological response and SVR in CHC patients. This suggests that strategies to improve IR may have a positive effect on SVR and may be early monitored. This contradiction may be referred to different ethnicity or genotyping of the virus as they use larger numbers of Egyptians and Spanish patients [22]. In Ziada and colleagues' study, a total of 140 chronic HCV Egyptian patients were divided into two groups according to the homeostasis model assessment-IR (HOMA-IR). Group 1 consisted of 48 chronic HCV patients with HOMA-IR $\geqslant 2$, and group 2 consisted of 92 chronic HVC patients without IR (HOMA IR < 2). All patients were treated with combination therapy (pegylated interferon-alpha 2a plus ribavirin) for 48 weeks and studied for viral kinetics throughout the period of therapy [23]. They concluded that IR in chronic HCV patients is associated with progressive fibrosis and slow viral kinetics, and could be a predictor for lack of rapid and sustained virological response. Therefore, HOMA-IR levels should be measured and improved before starting antiviral treatment. The contradiction between the current study and Ziada and colleagues' study was due to larger number of the studied patients and in Ziada and colleagues' study they use SVR instead of EVR.

Cammá and colleagues [24] reported that moderate/severe steatosis and not IR are associated with low likelihood of SVR in G1 HCV patients. Also in a study conducted on 155 HCV/HIV co infected patients at high prevalence of IR, IR was not predictor of SVR to pegylated interferon plus ribavirin This study identifies HCV genotype, viral load and baseline LDL cholesterol levels as independent predictors of SVR but the impact of steatosis was not evaluated [9].

On the other hand several other studies reported association between IR and virological response. Romero-Gomez and colleagues [7] demonstrated lower rate of SVR among genotype1 CHC patients with IR compared to those without IR (32.8% vs. 60.5%). Also Conjeevaram and colleagues [8] confirmed the independent association between IR and SVR rate in G1 African-American and White-American infected

patients with a mean HOMA value ranging from 3.5 to 6.8. Also in Asiatic patients with G1 CHC, IR, but not steatosis, was an independent predictor of poor response to antiviral treatment [25].

A similar result was reported in obese HCV patient with genotypes 2 and 3 [19] and a recent study on genotype 4 reported significant association between IR and SVR, RVR [18].

The contradictory results may be related to the difference in the baseline characteristics of patients such as ethnicity, BMI, levels of serum cholesterol and triglycerides, degree of steatosis and viral factors such as viral genotype and baseline viral load.

In the present study, we did not find direct association between IR and hepatic fibrosis nor necroinflammation. This is similar to the result obtained by **Yoneda and colleagues** [26]. On the other hand other studies showed a link between hepatic fibrosis from CHC infection and IR, particularly for obese patients and those with severe fibrosis (stages 3–4) [27,28].

There was a direct correlation between IR and liver steatosis. Consequently, there was a significant correlation between liver steatosis, and score of liver inflammation and grades of liver fibrosis. So, we may deduce that IR has an indirect effect on liver inflammation and liver fibrosis.

This lack of direct association may be due to the fact that the global level of insulin resistance is likely to depend on the contribution from the adipose tissue and the muscle, two extra hepatic compartments that are not infected by HCV. This result may suggest that the IR in HCV-infected patients may be independent of hepatic inflammation or fibrosis.

In the current study IR was not associated with viral load which is contradictory to previous studies that showed association of IR with HCV RNA level [4,25,29,30].

The relation between HCV and IR is a complex one it is not evident whether IR stimulates viral replication or HCV increases IR. Hepatitis C core protein promotes degradation of insulin receptor substrate-1(IRS1) and induces insulin resistance. The up regulation of IL6 and TNFα in CHC cause reduction of hepatic insulin receptor autophosphorylation and phosphorylation of IRS1 and 2 in cell culture and mice and interfere with the insulin signaling cascade [5,31,32]. On the other hand, IR promotes hepatic inflammation and fibrosis by inducing steatosis [33].

5. Conclusion

Insulin resistance is a common finding in CHC, it is associated with increase liver steatosis. However it has no impact on EVR to combined interferon ribavirin therapy, viral load or necroinflammation.

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